

AMENDMENTS TO THE CLAIMS

Please amend the claims as noted below, without prejudice to subsequent renewal. The listing of claims below replaces all prior versions, and listings, of claims in the application.

These amendments introduce no new matter and support for the amendment is replete throughout the specification and claims as originally filed. These amendments are made without prejudice and are not to be construed as abandonment or dedication of the previously claimed subject matter, or agreement with any objection or rejection of record.

Listing of Claims:

1. (Original) A composition, comprising: a cell comprising a caged sensor for detecting an activity of an enzyme, which caged sensor comprises:
 - a) one or more molecules collectively comprising:
 - i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and
 - ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,
 - b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate.
2. (Original) A composition, comprising: a caged sensor for detecting an activity of an enzyme, which caged sensor comprises:
 - a) one or more molecules collectively comprising:
 - i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate.

3. (Original) The composition of claim 1 or 2, wherein the first caging groups inhibit the enzyme from acting upon the substrate by at least about 75%, at least about 90%, at least about 95%, or at least about 98%, as compared to the substrate in the absence of the first caging groups.

4. (Original) The composition of claim 1 or 2, wherein the first caging groups prevent the enzyme from acting upon the substrate.

5. (Original) The composition of claim 1 or 2, wherein removal of, or an induced conformational change in, the first caging groups permits the enzyme to act upon the substrate.

6. (Original) The composition of claim 1 or 2, wherein the first label is an optically detectable label or a fluorophore; wherein the first signal and/or the second signal is an optical signal, a fluorescent signal, a luminescent signal, a nonoptical signal, or a magnetic signal; or wherein the first signal is a fluorescent emission at a first wavelength with a first intensity and the second signal is a fluorescent emission at the first wavelength with a second intensity substantially greater or less than the first intensity.

7. (Original) The composition of claim 1 or 2, wherein the one or more first caging groups associated with the one or more molecules are covalently attached to the one or more molecules.

8. (Original) The composition of claim 1 or 2, wherein the one or more first caging groups are removable by sonication, photoactivatable, or photolabile; or wherein the first caging groups can be removed by exposure to light with a wavelength between about 60 nm and about 400 nm, between about 400 nm and about 700 nm, and/or between about 700 nm and about 1000 nm.

9. (Original) The composition of claim 1 or 2, wherein the first label and the substrate are physically connected.
10. (Original) The composition of claim 1 or 2, wherein the substrate comprises one or more of: an amino acid, a polypeptide, a nitrogenous base, a nucleoside, a nucleotide, a nucleic acid, a carbohydrate, or a lipid.
11. (Original) The composition of claim 2, comprising the enzyme, a cell, a cell comprising the caged sensor, a cell comprising the enzyme, or a cell lysate.
12. (Original) The composition of claim 2, wherein the enzyme is an oxidoreductase, a transferase, a hydrolase, a lyase, a ligase, or an isomerase.
13. (Original) The composition of claim 1, wherein the enzyme is an oxidoreductase, a transferase, a hydrolase, a lyase, a ligase, an isomerase, a phosphatase, a GTPase, an ATPase, a phosphodiesterase, a luciferase, an acetylase, a glycosylase, a ubiquitin-conjugating enzyme, a hydrogenase, a polymerase, a peroxidase, a protease, or a caspase.
14. (Original) The composition of claim 13, wherein the enzyme is a caspase, and wherein one polypeptide comprises the substrate for the caspase and the first label and comprises a second label or a quencher; wherein the first label and the second label or the quencher interact to produce the first signal when the substrate is intact; and wherein cleavage of the substrate by the caspase prevents the interaction of the first label and the second label or the quencher, thereby resulting in production of the second signal.
15. (Original) The composition of claim 14, wherein the caspase is caspase 3, the substrate comprises an Asp-Glu-Val-Asp motif, and the one or more first caging groups are located on one or more of the amino acid residues in the Asp-Glu-Val-Asp motif.
16. (Original) The composition of claim 14, wherein one of the first label and the second label or the quencher is located at the N-terminus of the polypeptide and the other of the first label and the second label or the quencher is located at the C-terminus of the polypeptide.
17. (Original) The composition of claim 14, wherein the first and second labels are fluorophores capable of exhibiting FRET, or wherein the first label is FITC and the second label is rhodamine or coumarin.

18. (Original) The composition of claim 1 or 2, wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine.

19. (Previously presented) The composition of claim 18, wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the first label and the substrate for the kinase, the substrate comprising a serine, threonine, or tyrosine residue capable of being phosphorylated by the kinase; wherein the first label is located at the serine, threonine, or tyrosine residue and exhibits the first signal when the residue is not phosphorylated and the second signal when the residue is phosphorylated.

20. (Canceled)

21. (Currently amended) ~~The composition of claim 18;~~ A composition, comprising:

1) a cell comprising a caged sensor for detecting an activity of an enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; or

2) a caged sensor for detecting an activity of an enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,
b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate;
wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label and comprises a second label or a quencher; wherein the first label and the second label or the quencher interact to produce the first signal when the substrate is not phosphorylated; and wherein phosphorylation of the substrate prevents the interaction of the first label and the second label or the quencher, thereby resulting in production of the second signal.

22. (Previously presented) The composition of claim **21**, wherein the one or more first caging groups are located on a residue that can be phosphorylated by the kinase.

23. (Original) The composition of claim **21**, wherein one of the first label and the second label or the quencher is located at the N-terminus of the polypeptide and the other of the first label and the second label or the quencher is located at the C-terminus of the polypeptide.

24. (Original) The composition of claim **21**, wherein the first and second labels are hydrophobic fluorophores, or wherein the first label is FITC and the second label is rhodamine or coumarin.

25. (Original) The composition of claim **21**, wherein phosphorylation of the substrate triggers a conformational change in the polypeptide, the conformational change preventing the interaction of the first label and the second label or the quencher; or wherein phosphorylation of the substrate results in binding of a phosphobinder to the phosphorylated substrate, the binding of the phosphobinder preventing the interaction of the first label and the second label or the quencher.

26. (Original) The composition of claim 25, wherein the phosphobinder is associated with one or more second caging groups, the presence of which prevents the phosphobinder from binding the phosphorylated substrate.

27. (Original) The composition of claim 26, wherein the second caging groups are removable under different conditions than the first caging groups preventing phosphorylation of the substrate.

28. (Original) The composition of claim 25, wherein the phosphobinder comprises an antibody, an SH-2 domain, a PTB domain, a 14-3-3 domain, an FHA domain, a WD40 domain and/or a WW domain.

29. (Currently amended) ~~The composition of claim 18,~~ A composition, comprising:

1) a cell comprising a caged sensor for detecting an activity of an enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; or

2) a caged sensor for detecting an activity of an enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,
b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate;
wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase and the first label; wherein the polypeptide comprises a phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in intramolecular binding of the phosphobinder to the phosphorylated substrate, the intramolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal; or, wherein the one or more molecules comprise a first polypeptide and a second polypeptide; wherein the first polypeptide comprises the substrate for the kinase and the first label; wherein the second polypeptide comprises a phosphobinder and a second label or a quencher; wherein the first label and the second label or the quencher do not interact when the substrate is not phosphorylated, thereby producing the first signal; and wherein phosphorylation of the substrate results in intermolecular binding of the phosphobinder to the phosphorylated substrate, the intermolecular binding resulting in the interaction of the first label and the second label or the quencher, thereby producing the second signal.

30. (Previously presented) The composition of claim **29**, wherein the one or more first caging groups are located on a residue that can be phosphorylated by the kinase.

31. (Original) The composition of claim **29**, wherein one of the first label and the second label or the quencher is located at the N-terminus of the polypeptide and the other of the first label and the second label or the quencher is located at the C-terminus of the polypeptide.

32. (Original) The composition of claim 29, wherein the first and second labels are fluorophores capable of exhibiting FRET.

33. (Original) The composition of claim 29, wherein the phosphobinder is associated with one or more second caging groups, the presence of which prevents the phosphobinder from binding the phosphorylated substrate.

34. (Original) The composition of claim 33, wherein the second caging groups are removable under different conditions than the first caging groups preventing phosphorylation of the substrate.

35. (Original) The composition of claim 29, wherein the phosphobinder comprises an antibody, an SH-2 domain, a PTB domain, a 14-3-3 domain, an FHA domain, a WD40 domain and/or a WW domain.

36. (Currently amended) ~~The composition of claim 18,~~ A composition, comprising:

1) a cell comprising a caged sensor for detecting an activity of an enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate; or

2) a caged sensor for detecting an activity of an enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state,

wherein the first state is not converted to the second state by cleavage by the enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,

b) one or more first caging groups associated with the one or more molecules, the first caging groups inhibiting the enzyme from acting upon the substrate;

wherein the enzyme is a protein kinase that phosphorylates tyrosine, serine and/or threonine;

wherein the one or more molecules comprise one polypeptide; wherein the one polypeptide comprises the substrate for the kinase, a second substrate, the first label, a third label, a fourth label or a quencher, and a phosphobinder; the substrate comprising a serine, threonine, or tyrosine residue capable of being phosphorylated by the kinase; the second substrate being associated with one or more third caging groups, the presence of which prevents phosphorylation of the second substrate; wherein the first label is located at the serine, threonine, or tyrosine residue and exhibits the first signal when the residue is not phosphorylated and the second signal when the residue is phosphorylated; wherein the third label and the fourth label or the quencher do not interact when the second substrate is not phosphorylated, thereby producing a third signal; and wherein phosphorylation of the second substrate results in intramolecular binding of the phosphobinder to the phosphorylated second substrate, the intramolecular binding resulting in the interaction of the third label and the fourth label or the quencher, thereby producing a fourth signal, the fourth signal distinguishable from the first, second and third signals.

37. (Original) The composition of claim 36, wherein the second substrate is for the same kinase or for a different kinase.

38. (Previously presented) The composition of claim 36, wherein the one or more third caging groups are located on a residue that can be phosphorylated by the kinase.

39. (Original) The composition of claim 38, wherein the third caging groups preventing phosphorylation of the second substrate are removable under different conditions than the first caging groups preventing phosphorylation of the substrate.

40. (Original) The composition of claim 36, wherein one of the third label and the fourth label or the quencher is located at the C-terminus of the polypeptide and the other of the third label and the fourth label or the quencher is within the polypeptide.

41. (Original) The composition of claim 36, wherein the third and fourth labels are fluorophores capable of exhibiting FRET.

42. (Original) The composition of claim 36, wherein the phosphobinder is associated with one or more second caging groups, the presence of which prevents the phosphobinder from binding the phosphorylated second substrate.

43. (Original) The composition of claim 42, wherein the second caging groups are removable under different conditions than the first caging groups preventing phosphorylation of the substrate and/or under different conditions than the third caging groups preventing phosphorylation of the second substrate.

44. (Original) The composition of claim 36, wherein the phosphobinder comprises an antibody, an SH-2 domain, a PTB domain, a 14-3-3 domain, an FHA domain, a WD40 domain and/or a WW domain.

45. (Currently amended) ~~The composition of claim 1 or 2,~~ A composition, comprising:

1) a cell comprising a caged sensor for detecting an activity of an enzyme, which caged sensor comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and

ii) a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state, and,

b) one or more first caging groups associated with the one or more molecules,
the first caging groups inhibiting the enzyme from acting upon the substrate, or
2) a caged sensor for detecting an activity of an enzyme, which caged sensor
comprises:

a) one or more molecules collectively comprising:

i) a substrate for the enzyme, wherein the substrate is in a first state on
which the enzyme can act, thereby converting the substrate to a second state,
wherein the first state is not converted to the second state by cleavage by the
enzyme, and

ii) a first label, wherein a first signal exhibited by the first label when the
substrate is in its first state is distinguishable from a second signal exhibited by
the first label when the substrate is in its second state, and,

b) one or more first caging groups associated with the one or more molecules,
the first caging groups inhibiting the enzyme from acting upon the substrate;

wherein the one or more molecules comprise a fifth label, the fifth label exhibiting a unique fifth signal, the fifth signal being independent of the state of the substrate.

46. (Original) The composition of claim 45, wherein the fifth label is a fluorophore or a quantum dot.

47. (Original) The composition of claim 1 or 2, wherein the one or more molecules are associated with a cellular delivery module that can mediate introduction of the sensor into a cell.

48. (Original) The composition of claim 47, wherein the cellular delivery module comprises a polypeptide, a PEP-1 peptide, an amphipathic peptide, a cationic peptide, a protein transduction domain, a protein transduction domain derived from an HIV-1 Tat protein, from a herpes simplex virus VP22 protein, or from a Drosophila antennapedia protein, or a model protein transduction domain.

49. (Original) The composition of claim 47, wherein the cellular delivery module is covalently attached to the one or more molecules.

50. (Original) The composition of claim **49**, wherein the covalent attachment is reversible by exposure to light of a preselected wavelength.

51. (Original) The composition of claim **47**, wherein the cellular delivery module is associated with one or more fourth caging groups, the presence of which prevents the cellular delivery module from mediating introduction of the sensor into a cell.

52. (Original) The composition of claim **1** or **2**, wherein the one or more molecules are associated with at least one subcellular delivery module.

53. (Original) The composition of claim **52**, wherein the subcellular delivery module comprises a polypeptide, a nucleic acid, and/or a carbohydrate; wherein the subcellular delivery module mediates localization of the sensor to one or more of: a membrane, a mitochondrion, a peroxisome, a nucleus, an endoplasmic reticulum, a Golgi, a vesicle, a lysosome, an endosome, or a chloroplast; wherein the subcellular delivery module comprises one or more of: a mitochondrial matrix-targeting sequence, a nuclear localization signal, a signal peptide, an ER retention signal, a peroxisomal targeting motif, a chloroplast stromal targeting sequence, a transmembrane domain, or a lipid attachment site; or wherein the subcellular delivery module comprises a binding domain that mediates localization of the sensor by binding to a target protein.

54. (Original) The composition of claim **52**, wherein the subcellular delivery module is covalently attached to the one or more molecules.

55. (Original) The composition of claim **54**, wherein the covalent attachment is reversible by exposure to light of a preselected wavelength.

56. (Original) The composition of claim **52**, wherein the subcellular delivery module is associated with one or more fifth caging groups, the presence of which prevents the subcellular delivery module from mediating subcellular localization of the sensor.

57. (Original) The composition of claim **2**, wherein the caged sensor is bound to a matrix.

58. (Original) The composition of claim **57**, wherein the matrix is a surface, and the sensor is bound to the surface at a predetermined location within an array comprising other sensors; or wherein the matrix comprises a bead.

59. (Previously presented) The composition of claim 1 or 2, wherein the caged sensor further comprises a first oligonucleotide, the first oligonucleotide being complementary to a second oligonucleotide, the second oligonucleotide being bound to a matrix.

60. (Original) The composition of claim 59, wherein the matrix is a surface, and the second oligonucleotide is bound to the surface at a predetermined location within an array comprising other oligonucleotides; or wherein the matrix comprises a bead.

61. (Original) A kit for making the caged sensor of claim 1 or 2, comprising a substrate, a first label, one or more first caging groups, and instructions for assembling the substrate, the first label, and the first caging groups to form the caged sensor, packaged in one or more containers; or comprising a first label, one or more first caging groups, and instructions for assembling the first label, the first caging groups, and a substrate supplied by a user of the kit to form the caged sensor, packaged in one or more containers.

62-200. (Cancelled).

201. (Withdrawn) A method of assaying an activity of an enzyme, the method comprising:
contacting the enzyme and a caged sensor by introducing the caged sensor into a cell,
the caged sensor comprising one or more molecules collectively comprising:

a) a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, and a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state, and

b) one or more caging groups associated with the one or more molecules, the caging groups inhibiting the enzyme from acting upon the substrate;

initiating the assay by exposing the enzyme and the caged sensor to uncaging energy of a first type, whereby exposure to the uncaging energy frees the sensor from inhibition by the first caging groups; and,

assaying the activity of the enzyme.

202. (Withdrawn) A method of assaying an activity of an enzyme, the method comprising:
contacting the enzyme and a caged sensor, the caged sensor comprising:

a) one or more molecules collectively comprising a substrate for the enzyme, wherein the substrate is in a first state on which the enzyme can act, thereby converting the substrate to a second state, wherein the first state is not converted to the second state by cleavage by the enzyme, and a first label, wherein a first signal exhibited by the first label when the substrate is in its first state is distinguishable from a second signal exhibited by the first label when the substrate is in its second state; and,

b) one or more caging groups associated with the one or more molecules, the caging groups inhibiting the enzyme from acting upon the substrate;

initiating the assay by exposing the enzyme and the caged sensor to uncaging energy of a first type, whereby exposure to the uncaging energy frees the sensor from inhibition by the first caging groups; and,

assaying the activity of the enzyme.

203. (Withdrawn) The method of claim **202**, wherein contacting the enzyme and the caged sensor comprises introducing the caged sensor into a cell.

204. (Withdrawn) The method of claim **201** or **203**, comprising introducing a vector encoding the enzyme into the cell.

205. (Withdrawn) The method of claim **201** or **202**, wherein the one or more caging groups prevent the enzyme from acting upon the substrate, and wherein removal of or an induced conformational change in the one or more caging groups permits the enzyme to act upon the substrate.

206. (Withdrawn) The method of claim **201** or **202**, wherein exposing the enzyme and the caged sensor to uncaging energy of a first type comprises sonicating the enzyme and the caged sensor or exposing the enzyme and the caged sensor to light of a first wavelength.

207. (Withdrawn) The method of claim **206**, wherein exposing the enzyme and the caged sensor to light of a first wavelength comprises exposing one or more preselected areas to the light.

208. (Withdrawn) The method of claim **207**, wherein the one or more preselected areas comprise one or more wells of a multiwell plate; wherein the one or more preselected areas comprise a plurality of the wells of a multiwell plate, and wherein exposing the preselected

areas to the light comprises exposing the plurality of wells to the light simultaneously; wherein the one or more preselected areas comprise at least about 12, at least about 24, at least about 48, at least about 96, at least about 384, or at least about 1536 wells of a multiwell plate; wherein the one or more preselected areas comprise one or more channels of a microfluidic chip; wherein the one or more preselected areas comprise a plurality of the channels of the microfluidic chip, and wherein exposing the preselected areas to the light comprises exposing the plurality of channels to the light simultaneously; wherein the one or more preselected areas comprise one or more spots of a microarray; wherein the one or more preselected areas comprise a plurality of the spots of the microarray, and wherein exposing the preselected areas to the light comprises exposing the plurality of spots to the light simultaneously; or, wherein the one or more preselected areas comprise one or more regions of a cell, a tissue, or a body of an organism.

209. (Withdrawn) The method of claim **206**, wherein exposing the enzyme and the caged sensor to light of a first wavelength comprises exposing the enzyme and the caged sensor to light wherein the intensity of the light and the duration of exposure to the light are controlled such that a first portion of the caged sensor is uncaged and a second portion of the caged sensor remains caged; comprising repeating the assay by exposing the enzyme and the caged sensor to light of the first wavelength again.

210. (Withdrawn) The method of claim **209**, wherein the first portion is a selected amount.

211. (Withdrawn) The method of claim **201** or **202**, comprising detecting the first and/or the second signal.

212. (Withdrawn) The method of claim **201** or **202**, wherein the caged sensor is bound to a matrix, or wherein the caged sensor comprises a first oligonucleotide, the first oligonucleotide being complementary to a second oligonucleotide, the second oligonucleotide being bound to a matrix.

213. (Withdrawn) The method of claim **201** or **202**, comprising contacting the enzyme and a second caged component comprising one or more second caging groups, and exposing the second caged component to uncaging energy of a second type, whereby exposure to the uncaging energy frees the second component from inhibition by the second caging groups.

214. (Withdrawn) The method of claim **213**, wherein the second caged component comprises a nucleoside triphosphate, ATP, a metal ion, a polypeptide, a nucleic acid, a carbohydrate, a lipid, a phosphobinder, or an antibody.

215. (Withdrawn) The method of claim **213**, wherein exposing the second caged component to uncaging energy of a second type comprises exposing the second caged component to light of a second wavelength, different from the first and/or third wavelength.

216. (Withdrawn) The method of claim **201** or **202**, comprising contacting the enzyme with a third caged component, wherein the third caged component comprises a third component required for termination of the assay and one or more third caging groups associated with the third component, the presence of the third caging groups inhibiting the third component from terminating the assay; and comprising exposing the third caged component to uncaging energy of a third type, whereby exposure to the uncaging energy frees the third component from inhibition by the third caging groups.

217. (Withdrawn) The method of claim **216**, wherein exposing the third caged component to uncaging energy of a third type comprises exposing the third caged component to light of a third wavelength, different from the first and/or second wavelength.

218. (Withdrawn) The method of claim **216**, wherein the third component comprises an inhibitor, a chelating agent, EGTA or EDTA.

219. (Withdrawn) The method of claim **201** or **202**, comprising contacting the enzyme with a fourth caged component, wherein the fourth caged component comprises a modulator and one or more fourth caging groups associated with the modulator; and comprising exposing the fourth caged component to uncaging energy of a fourth type, whereby exposure to the uncaging energy frees the modulator from inhibition by the fourth caging groups.

220. (Withdrawn) The method of claim **201** or **202**, comprising contacting a second enzyme with a fifth caged component, wherein the fifth caged component comprises a caged sensor for the second enzyme, the caged sensor comprising a sensor for the second enzyme and one or more fifth caging groups associated with the sensor; comprising exposing the fifth caged component to uncaging energy of a fifth type, whereby exposure to the uncaging energy frees the sensor from inhibition by the fifth caging groups.

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221. (Withdrawn) The method of claim **201** or **202**, wherein contacting the enzyme with one or more reagents comprises introducing the first caged component into a subcellular compartment, a tissue, or an organism.

222-303. (Cancelled).